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## DISTRIBUTION OF PORCINE CIRCOVIRUS 2 IN WILD BOAR IN ESTONIA

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**Background:** Porcine circovirus 2 (PCV2) causes post-weaning multi-systemic wasting syndrome and PCV2-related diseases. As according to literature wild boars are among the spreaders of virus, the aim of the study was to detect the spread of PCV2 in pigs in Estonia, detecting distribution areas of PCV2 in wild boars and comparing these with existing distribution areas of PCV2 in domestic pigs.

**Materials & Methods:** Samples of 1–4-year-old wild boars were selected from all Estonian counties. A total of 460 blood samples were examined with ELISA method (Ingezim Circo IgG) and randomly selected tissue sections from wild boar mesenteric lymph nodes by histopathological and immunohistochemical (IHC) studies. The organ samples were tested for PCV2 by RT-PCR and immunohistochemical stainings. For IHC, formalin-fixed, paraffin-embedded tissue sections were deparaffinized with xylene, rehydrated in alcohol and endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide. PCV2 monoclonal antibody (by Danish Institute for Food and Veterinary Research) 1:300 was used as primary antibody and EnVision+™ System (DakoCytomation) for incubation.

**Results:** Studies showed widespread distribution of PCV2 in wild boars in Estonia as ELISA test detected PCV2 antibodies in 92% of samples. Histopathological study revealed depletion of lymphocytes and proliferation of histiocytes. Locations of the most infected wild boars overlapped with locations of 96% of infected domestic pig farms.

**Conclusion:** Study revealed that domestic pig infection is higher in areas of higher wild boar infection. As the antibodies are present in 92% of animals exposed to PCV2, the probability that wild boars are spreading the virus to domestic pigs is high.

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BLUNT FORCE TRAUMA IN THE CANARIAN HOUBARA BUSTARD (*CHLAMYD-OTIS UNDULATA FUERTAVENTURAE*) PRODUCED BY COLLISION WITH OVERHEAD LINES

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**Background:** The mortality of birds resulting from collisions and electrocutions with overhead lines, such as power lines and phone lines among others, has been implicated in the decline of various avian species globally. Specifically, overhead line collisions pose a significant threat to the conservation of the Canarian houbara bustard (*Chlamydotis undulata fuertaventurae*), an endangered subspecies endemic to the Canary Islands.

**Materials & Methods:** This study centres on the post-mortem findings of Canarian houbara bustards that have collided with overhead lines, providing insights into the post-collision outcomes for these birds.

**Results:** A complete standardized necropsy of nine Canarian houbara bustards revealed that polytrauma was the cause of death in all cases. The most notable gross lesions associated with trauma included bone fractures, soft tissue lacerations, haemorrhages, luxations and haemocoelom, with certain body regions being more frequently affected. Histopathology, immunohistochemistry and entomology analysis confirmed that numerous birds survived the initial trauma, exhibiting variable survival intervals before succumbing to their injuries.

**Conclusion:** We concluded that when a houbara bustard collides with an overhead line it frequently survives the initial trauma. The histopathology, immunohistochemistry or entomologic analysis might be helpful to approximate the timing interval between trauma and death.

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PATHOLOGICAL STUDY OF A TRAUMATIC ANTHROPOGENIC INJURY IN THE SKELETON OF A SPINY BUTTERFLY RAY (*GYMNURA ALTAVELA*)

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**Background:** External injuries in elasmobranchs are frequent findings, either due to natural interactions or anthropogenic activities. However, there is a general lack of information about the pathological description of severe traumatic lesions. This work provides an overview of the clinical presentation, diagnostic imaging and pathological features of a traumatic skeletal injury in a spiny butterfly ray (*Gymnura altavela*).

**Materials & Methods:** An adult female was found lethargic on the seabed off the coast of Gran Canaria, with an external circular incised-contused traumatic lesion of 2 cm diameter in the scapulocoracoid cartilage. It was captured and transferred to the Poema del Mar Aquarium facilities for clinical evaluation and treatment options. Despite these efforts, the animal eventually died and was transferred to the IUSA for routine pathological diagnosis, including a computed tomography (CT) study.

**Results:** A notable reduction on the haematocrit and the hepatosomatic index confirmed a chronic debilitation process. The CT study revealed a comminuted fracture on the right scapulocoracoid cartilage, impacting the articular surfaces of the pectoral arch. The main pathological findings showed disorganization of the tesserae layer, macroscopically appearing as whitish square to rectangular geometric pieces. Histologically, these pieces of tesserae were separated from the unmineralized cartilage core and ripped out from the perichondrium. In the adjacent tissues, an intense infiltrate of granulocytes and fibrous connective tissue, oedema and haemorrhages were observed.

**Conclusion:** This study reports the first comprehensive description of skeleton trauma in a spiny butterfly ray, including the clinical presentation, diagnostic imaging and the anatomopathological features.

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DEADLY SERRATIA MARCESCENS INFECTION IN A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*)

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**Background:** Bacterial septicaemia is well described in dolphins and numerous bacterial genera, such as *Erysipelothrix*, *Streptococcus*, *Aeromonas*, *Pseudomonas*, etc., have been associated with it. *Serratia marcescens* is a gram-negative opportunistic pathogen, scarcely described in veterinary literature, which has been referred as an aetiological agent of nosocomial infections in humans, affecting the respiratory and urinary systems. This case report aims to describe the findings in a bottlenose dolphin (*Tursiops truncatus*), under human care, with a septicaemia due to *S. marcescens*.

**Materials & Methods:** A 21-year-old male bottlenose dolphin with clinical history of anorexia and renal azotaemia was brought for a full post-mortem study. Histopathology was performed and samples from blood, urine, liver and kidney were submitted for microbiology culture.

**Results:** On gross examination, the lungs presented multiple coalescent greyish-white nodules, and both kidneys showed multiple lobes with

areas of haemorrhage. The right ureter was distended and congested. Histopathology revealed a severe necrosuppurative pyelonephritis with gram-negative bacteria and bacterial emboli; a broncho-interstitial pneumonia with embolic foci was observed as well. In aerobic and anaerobic cultures, *S. marcescens* was isolated from blood, urine, kidney and lung. Other findings included a very chronic and depressed scar in the right pectoral fin, which precluded the dolphin from moving it properly. There were also tattoo-like lesions around the rostrum.

**Conclusion:** The pathological findings together with the isolation of *S. marcescens* in various tissues, including blood, are consistent with a bacterial sepsis. An ascending urinary infection is presumed to be the source of infection.

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#### CHARACTERIZATION OF AMYLOIDOSIS IN EURASIAN STONE-CURLEWS (*BURHINUS OEDICNEMUS*) FROM THE CANARY ISLANDS

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**Background:** Amyloid deposits consist of fibrils accumulation resulting from different precursor proteins. Their identification is crucial for adequate disease prognosis and treatment. In avian amyloidosis, most reports do not determine which fibrils are implicated or apply out-of-date methods. Other studies use antibody-based methods, but their use has important drawbacks, especially in scarcely studied species. Currently, laser microdissection followed by tandem mass spectrometry (LMD-MS/MS) is considered the gold standard for amyloid characterization.

**Materials & Methods:** Thirty specimens of Eurasian stone-curlews (*Burhinus oedicnemus*) were necropsied. Tissue samples were taken, routinely processed and stained with HE. When lesions consistent with amyloid deposit were observed, Congo red stain and polarized light were employed. After confirmation of amyloidosis in seven individuals, selected formalin-fixed paraffin embedded samples of five animals were processed and subjected to LMD-MS/MS.

**Results:** Seven Eurasian stone-curlews showed macroscopic and histological lesions consisting of amyloidosis, including hepatomegaly, splenomegaly, nephromegaly and the presence of extracellular amorphous hyaline deposits with a red discolouration when stained with Congo red and yellow-red-green birefringence under polarized light. Serum amyloid A (SAA) was the amyloid precursor protein most detected in all animals analysed but one, in which apolipoprotein A-I (ApoAI) was ampler. ApoAI and apolipoprotein A-IV (ApoAIV) were present in all samples. Heparan sulphate proteoglycan 2 (HSPG2) was present in four animals.

**Conclusion:** The amyloid proteome of the five specimens analysed consisted of SAA, ApoAI and ApoAIV and, frequently, HSPG2. ApoAI surpassed SAA in one case, consequently this lipoprotein should be considered as a potential precursor protein in avian species.

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#### HISTOPATHOLOGICAL CHANGES IN HUNTER-KILLED WILD BOARS NATURALLY INFECTED WITH AFRICAN SWINE FEVER VIRUS GENOTYPE II

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**Background:** Numerous studies have been published about lesions caused by African swine fever (ASF) virus Genotype II in naturally infected domestic pigs and experimentally infected animals. However, there is paucity of data regarding lesions caused by ASF virus in wild boars during the current ASF panzootic in Europe. The aim of this study was to determine

histopathological changes in hunter-killed wild boars that are PCR positive for ASF.

**Materials & Methods:** Tissues were selected from wild boars (n = 6) that were killed during active ASF surveillance in Latvia (2018–2023) and were PCR positive for ASF virus genome. HE-stained tissue sections of lymphoid organs (spleen, lymph node, tonsil) and kidneys were examined and scored according to a system adapted from the previously published protocol.

**Results:** Mild to moderately severe lesions characteristic of ASF were found in all animals. These included vascular thrombosis, tubular necrosis, haemorrhage and lymphoplasmacytic interstitial inflammation in the kidneys and lymphoid depletion in the lymphoid organs. Concurrently, lymphoid follicular hyperplasia was observed in lymph nodes and tonsils and extramedullary haematopoiesis was observed in the spleen. In 2/6 animals infiltration of multinucleated giant cells was found in the lymph nodes possibly indicating concurrent infection with porcine circovirus type 2.

**Conclusion:** All six hunter-killed wild boars positive for ASF virus genome had mild to moderate lesions characteristic of ASF in the kidneys and lymphoid organs. These lesions may represent an early clinical or sub-clinical stage of natural ASF infection. In some animals additional background lesions were detected suggestive of infection with other pathogens.

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#### FATAL NECROTIC-HAEMORRHAGIC PANCREATITIS ASSOCIATED WITH *VIBRIO HARVEYI* INFECTION IN A SPINY BUTTERFLY RAY (*GYMNURA ALTAVELA*)

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**Background:** Pancreatitis is a common mild finding in dead elasmobranchs, mostly attributed to parasite migration. However, it is rarely associated with severe clinical symptoms or as a primary cause of death. To date, no bacterial agents have been clearly associated with fatal pancreatitis in elasmobranchs.

**Materials & Methods:** An adult male spiny butterfly ray (*Gymnura altavela*), housed in a private aquarium under human care, showed non-specific signs of weakness. Despite treatment, the animal's condition deteriorated rapidly and, following its death, it was referred to IUSA for pathological investigation. Samples were taken for histological study and bacterial culture.

**Results:** The main macroscopic finding was a focal (2–3 cm) greenish-white necrotic area in the pancreas. Cutting this lesion revealed a purulent exudate. In addition, scattered white foci were observed throughout the pancreatic tissue. Microscopically, extensive areas of pancreatic necrosis with severe inflammatory reaction and haemorrhage were observed. Moreover, multifocal infiltrations of inflammatory cells (granulocytes and macrophages) were present in the interacinar spaces. *Vibrio harveyi* was isolated from a sample of the pancreas.

**Conclusion:** This work describes the histopathological findings of a severe necrotic-haemorrhagic pancreatitis and its association with *V. harveyi* infection as a potential cause of death in a spiny butterfly ray.

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#### PLASTIC CONTAMINATION AND LESIONS OBSERVED IN CORY'S SHEARWATERS (*C. BOREALIS*) FROM THE AZORES ARCHIPELAGO - A PILOT STUDY

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